

1 TTTCCCTCACTATAAAGAATAGAGAAAGGAGGCTTCAGTGACCGGCTGCCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGATC
1 ATGGCTATGATGGAGGTCACAGGGGAGCCAGCCTGGGACAGACCTGCGTGCTGATCGTGATCTTACAGTGCTCCTGCAGTCTCTCTGT
1 MetAlaMetMetGluValGlnGlyGlyProSerLeuGlyGlnThrCysValLeuIleValIlePheThrValLeuLeuGlnSerLeuCys
181 GTGGCTGTAACTTACGTGTACTTTACCAACGAGCTGAAGCAGATGCAGGACAAGTACTCCAAAAGTGCGCATTTGCTTTCTTAAAGAA
31 ValAlaValThrTyrValTyrPheThrAsnGluLeuLysGlnMetGlnAspLysTyrSerLysSerGlyIleAlaCysPheLeuLysGlu
271 GATGACAGTTATTGGGACCCCAATGACGAAGAGAGTATGAACAGCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAG
61 AspAspSerTyrTrpAspProAsnAspGluGluSerMetAsnSerProCysTrpGlnValLysTrpGlnLeuArgGlnLeuValArgLys
361 ATGATTTTGCAGAACCTCTGAGGAAACCATTTCTACAGTTCAAGAAAGCAACAAAATATTTCTCCCTAGTGAGAGAAAGAGGTCCNCAG
91 MetIleLeuArgThrSerGluGluThrIleSerThrValGlnGluLysGlnGlnAsnIleSerProLeuValArgGluArgGlyProGln
451 AGAGTAGCAGCTCACATAACTGGGACCCAGAGGAAGAACACACATTGTCTTCTCCAAACTCCAAAGAAATGAAAAGGCTCTGGGCGGCAAA
121 ArgValAlaAlaHisIleThrGlyThrArgGlyArgSerAsnThrLeuSerSerProAsnSerLysAsnGluLysAlaLeuGlyArgLys
541 ATAAACTCCTGGGAATCATCAAGGAGTGGGCATTTCATTCCTGAGCAACTTGCACTTGAGGAATGGTGAACCTCCATCCATGAAAAAGGG
151 IleAsnSerTrpGluSerSerArgSerGlyHisSerPheLeuSerAsnLeuHisLeuArgAsnGlyGluLeuValIleHisGluLysGly
631 TTTTACTACATCTATTCCCAACACATACTTTTCGATTTTCAGGAGGAATAAAGAAAACACAAAAGACGACAAACAATGGTCCCAATATATT
181 PheTyrTyrIleTyrSerGlnThrTyrPheArgPheGlnGluIleLysGluAsnThrLysAsnAspLysGlnMetValGlnTyrIle
721 TACAAATACACAAAGTTATCCTGACCCCTATATTGTTGATGAAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGAATATGGACTCTAT
211 TyrLysTyrThrSerTyrProAspProIleLeuLeuMetLysSerAlaArgAsnSerCysTrpSerLysAspAlaGluTyrGlyLeuTyr
811 TCCATCTATCAAGGGGAATATTTGAGCTTAAGGAAATGACAGAAATTTTGTGTTCTGTAACAAAATGAGCACCTTGATAGACATGGACCAT
241 SerIleTyrGlnGlyIlePheGluLeuLysGluAsnAspArgIlePheValSerValThrAsnGluHisLeuIleAspMetAspHis
901 GAAGCCAGTTTTTTCGGGGCCTTTTGTAGTTGGCTAACTGACCTGGAAAGAAAAGCAATAACCTCAAAGTGACTATTTCAGTTTTTCAGGAT
271 GluAlaSerPhePheGlyAlaPheLeuValGlyStp
991 GATACACTATGAAGATGTTTCAAAAAAATCTGACCAAAACAAACACAGAAA

FIG. 1

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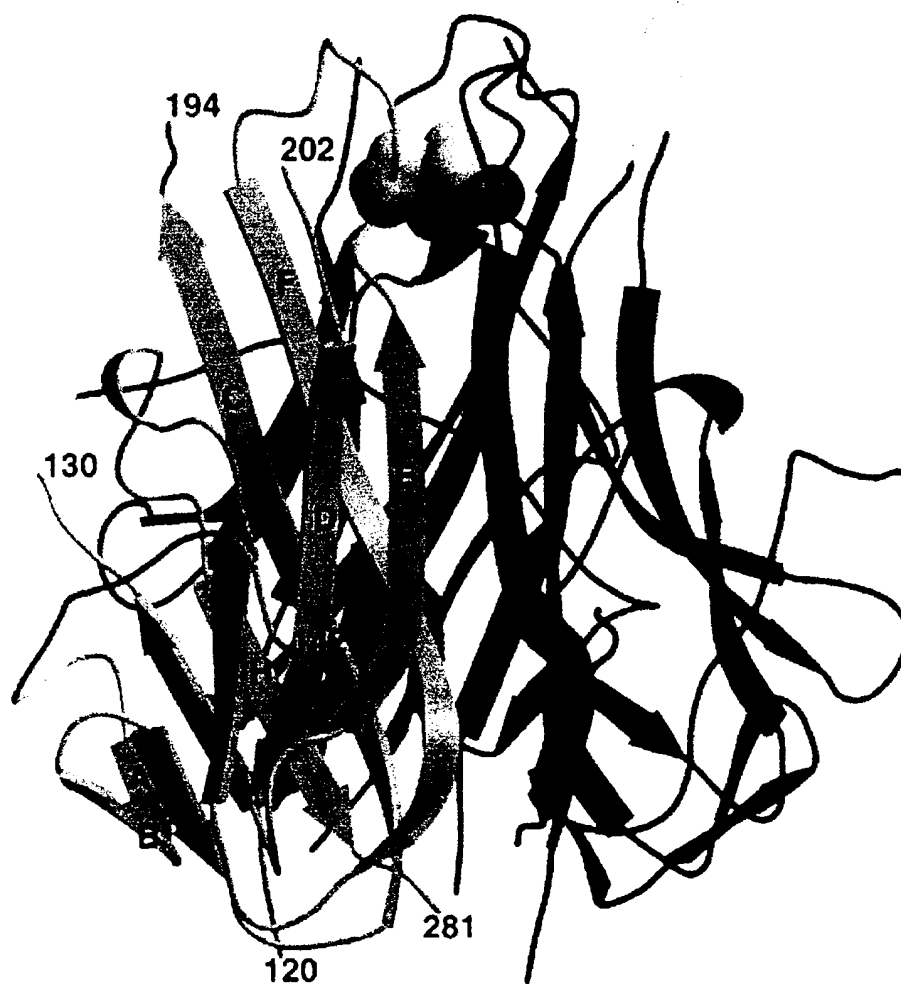


FIG. 2A

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FIG._2B



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Crystallographic Data

	<u>Apo-2L (114-281)</u>	<u>Apo-2L (91-281) D218A</u>	<u>Apo-2L (91-281) D218A</u>
Crystal			
Space Group	P6 ₃	R32	R32
Unit Cell (Å)	a=72.5 c=140	a=66.4 c=197.6	a=66.4 c=197.7
Resolution (Å)	3.9	1.9	1.3
Coverage (%)	94 (96)	93 (99)	100 (100)
<I/σ(I)>	5.9	10.1	12.4
# Unique (hkl)	3589	12680	41840
Redundancy	4.9	4.3	12.1
R _{symm} (%)	15.4 (34)	6.2 (27)	6.4 (34)
# Protomers in ASU	2	1	1
Refinement			
R _{cryst} (%)	33.8	20	
R _{free} (%)	27.6	22	
rmsd Bonds (Å)	0.009	0.015	0.007
rmsd Angles (°)	1.79	2.0	1.41
Average B-Values	—	14	14
# Water Molecules	0	170	

$R_{\text{symm}} = \frac{\sum_h \sum_i (I_{hi} - \langle I_h \rangle)}{\sum_h I}$ where I_h is the mean structure factor intensity of i observations of symmetry-related reflections with Bragg index h . $R_{\text{cryst}} = \frac{\sum_h \sum_i |F_{\text{obs}}| - |F_{\text{calc}}|}{\sum_i |F_{\text{obs}}|}$ where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes. $R_{\text{free}} = \frac{\sum_{(hkl) \in \tau} |F_{\text{obs}}(hkl)| - k |F_{(hkl)}|}{\sum_{(hkl) \in \tau} |F_{\text{obs}}(hkl)|}$ where the τ set of reflections is omitted from the refinement process. 10% of the data were included in the τ set for calculation of R_{free} and not included in refinement. Values in parenthesis are for the highest resolution shell.

FIG. 2C

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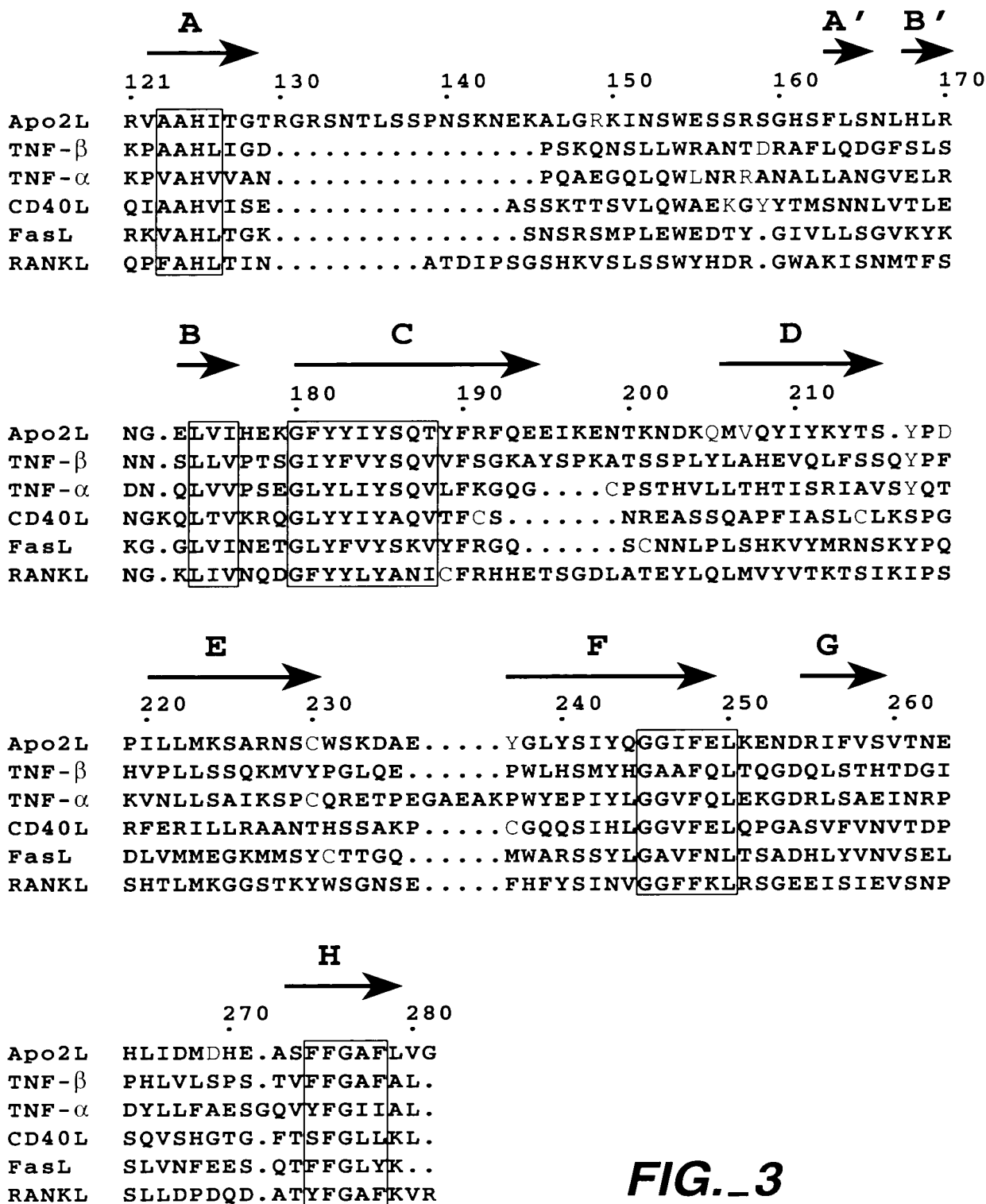


FIG. 3



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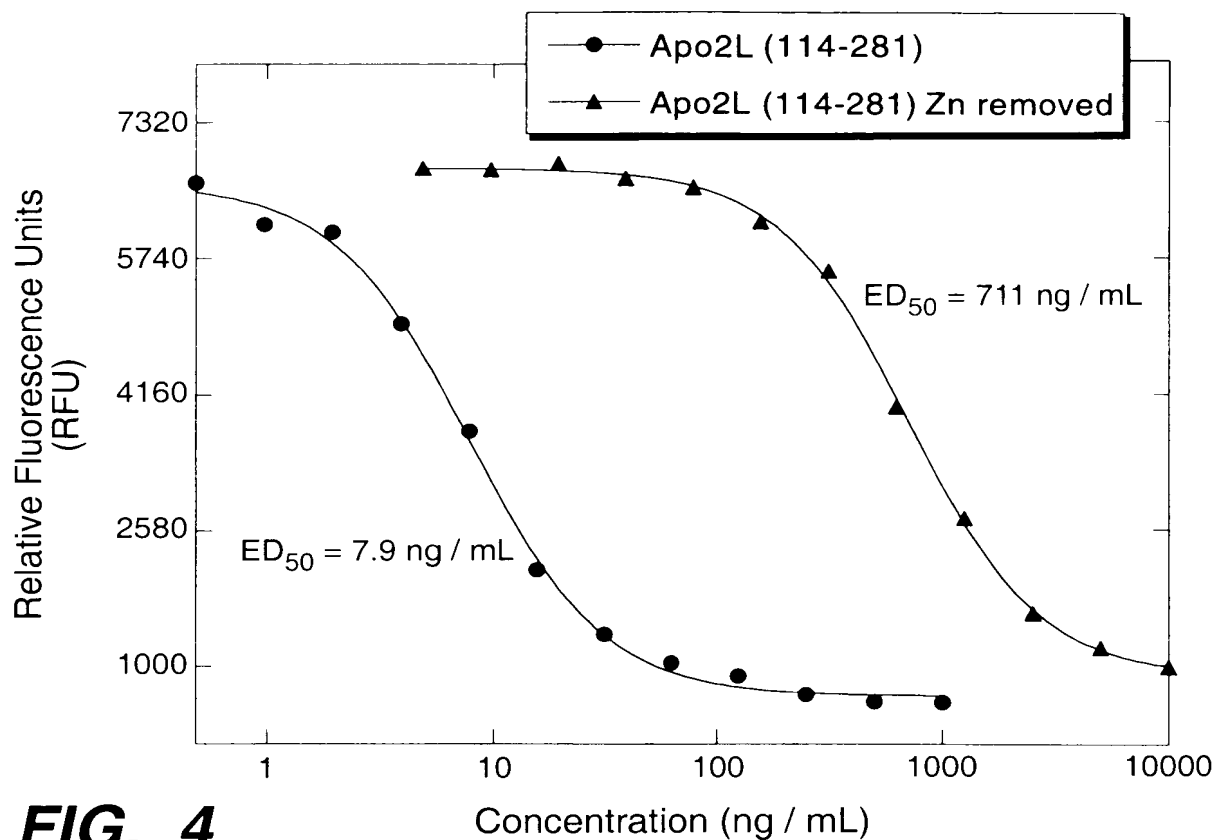


FIG._4

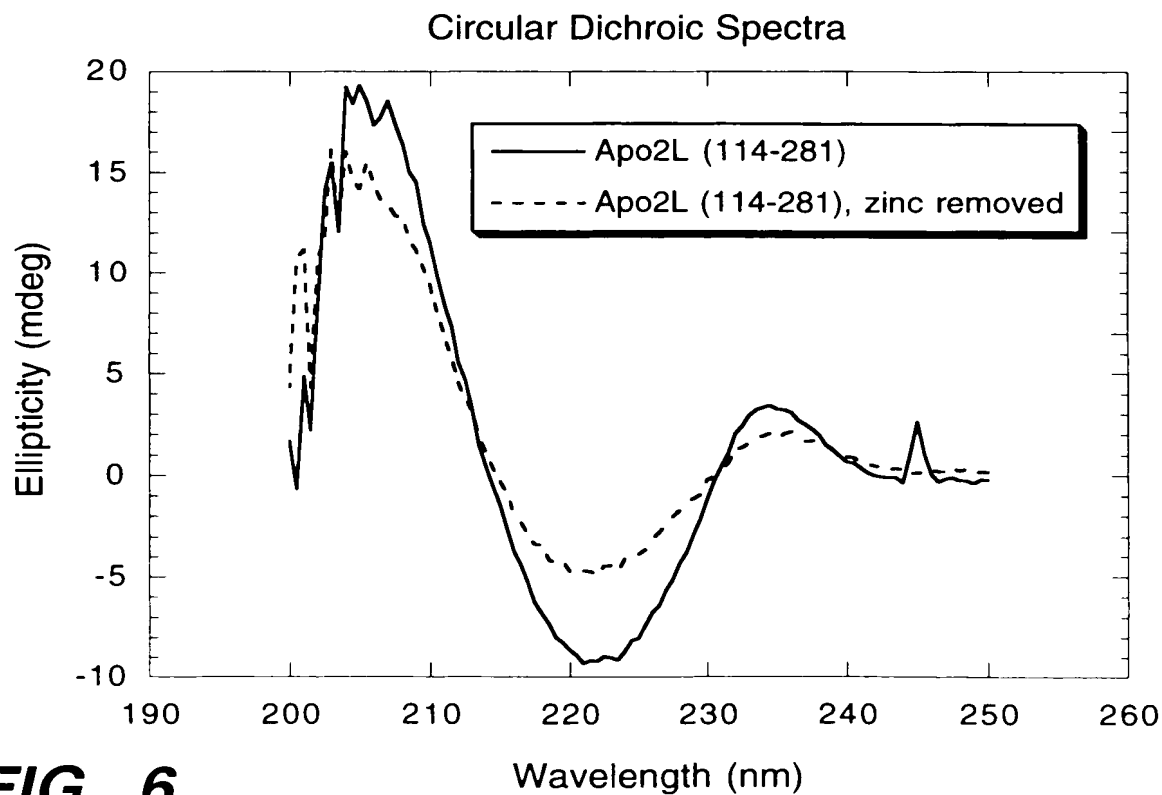


FIG._6

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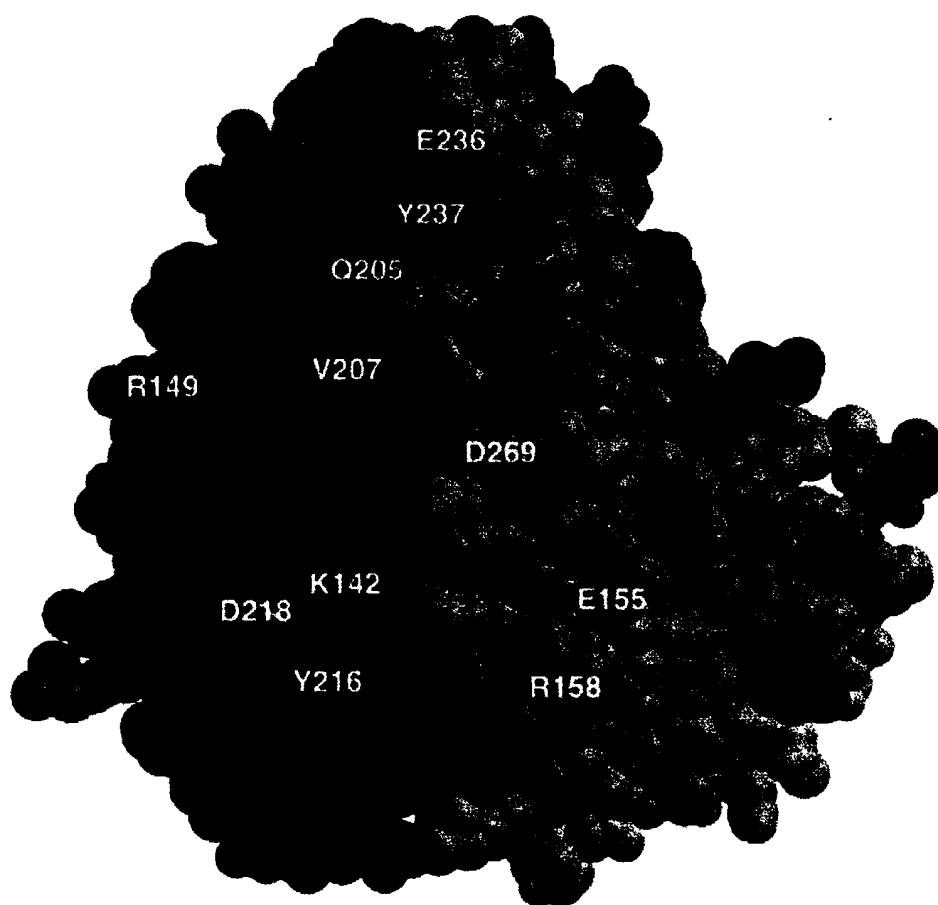


FIG. 5



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Effect of Zn Removal On Stability: Thermal Denaturation By CD

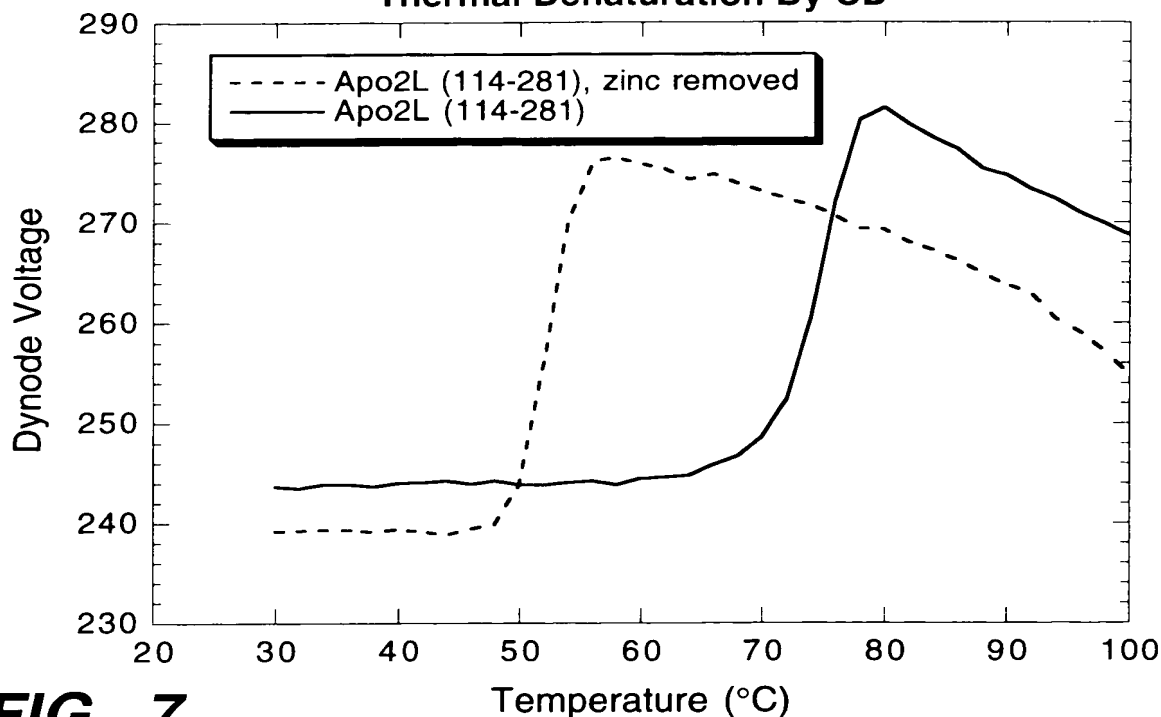


FIG._7

Effect of ZnSO₄ Additions On Apo2L Product Accumulation

Production Host: 43E7
(E. coli W3110 fhuA (tonA) phoA Δ(argF-lac) degP kanS ptr3 ompT llvG+)

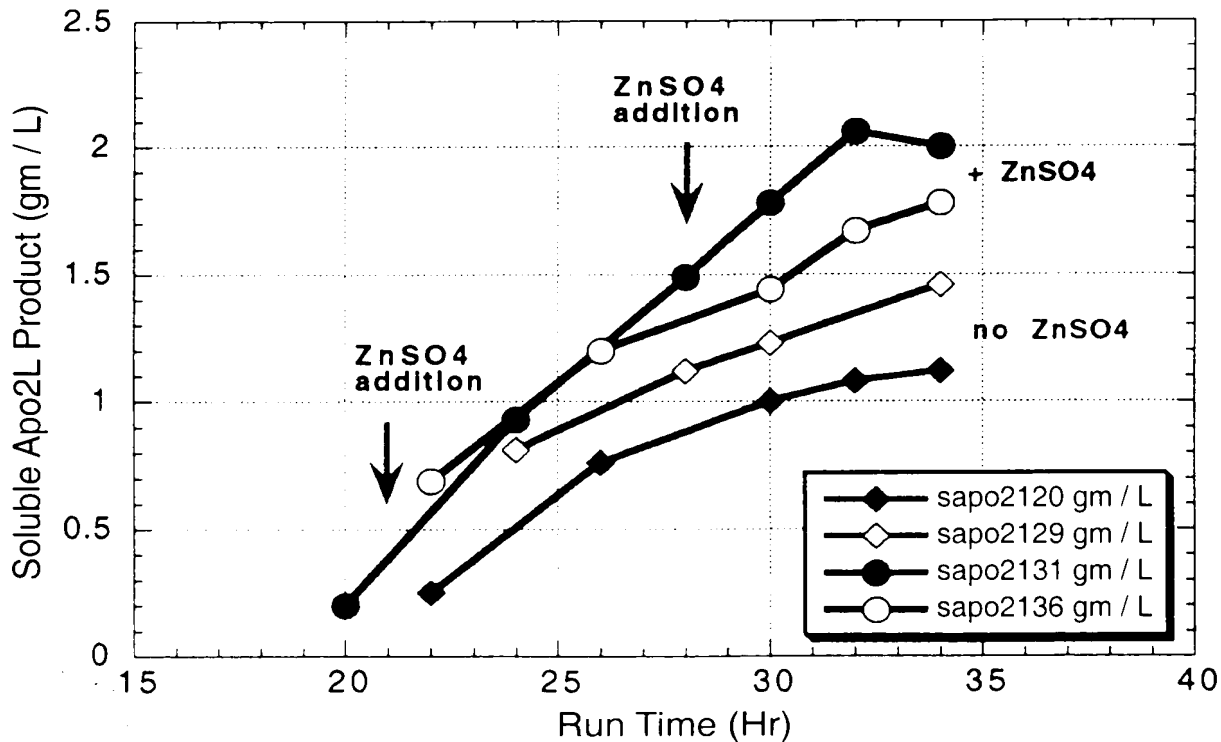


FIG._8

AP-Apo2L +/- ZnSO₄ gm / L

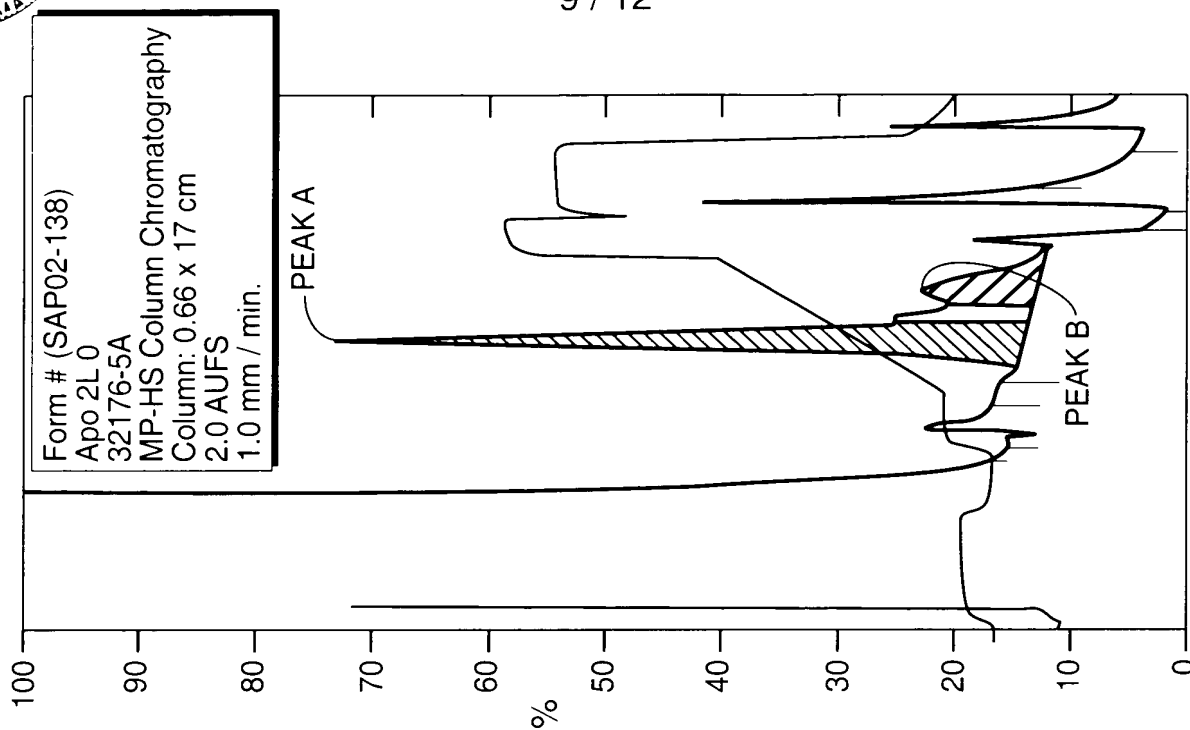
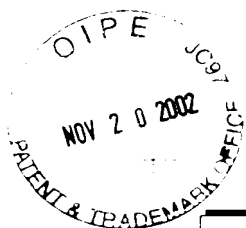


FIG. 9B

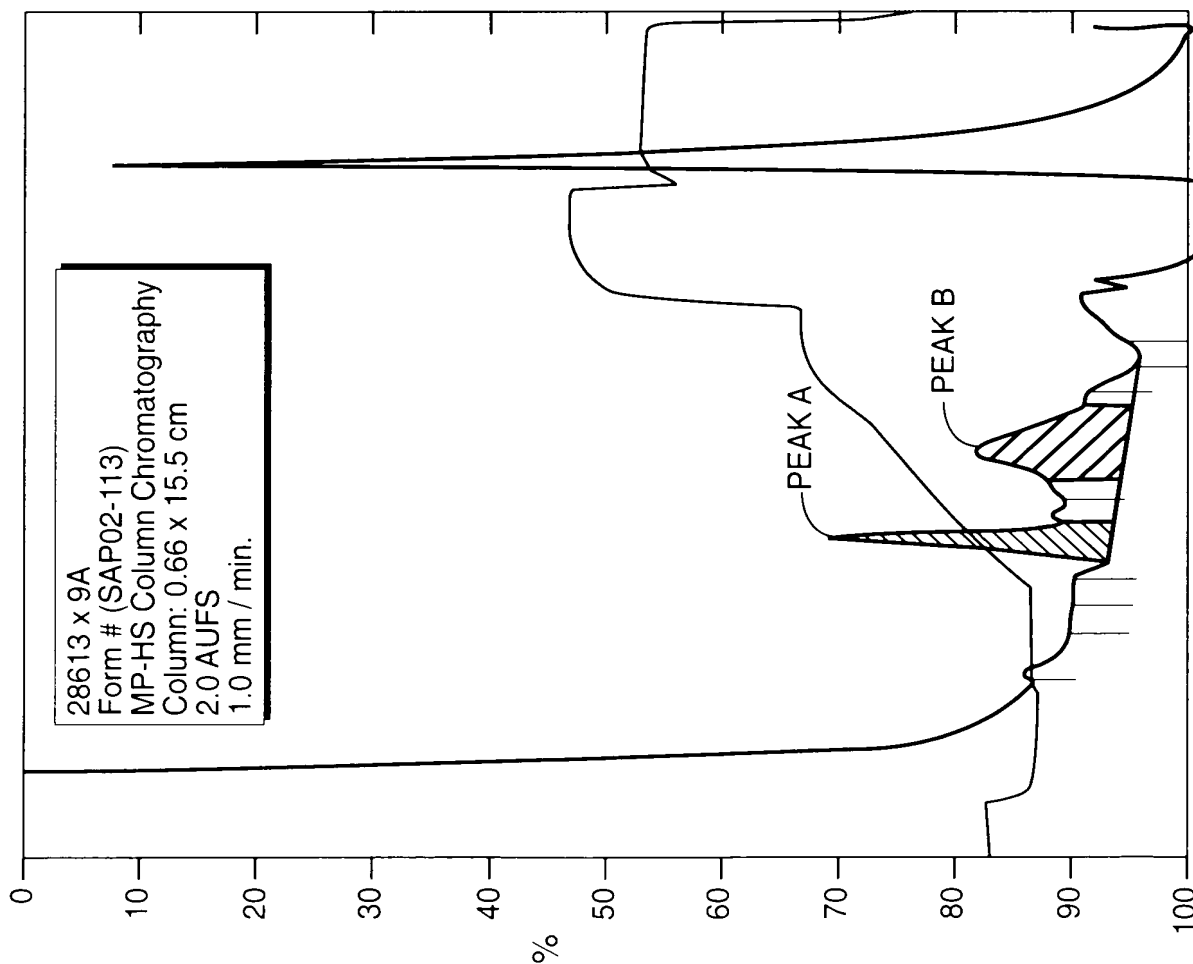


FIG. 9A

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Effect of ZnSO₄ Additions On Apo2L Product Accumulation Using trp Promoter For Expression by E. coli

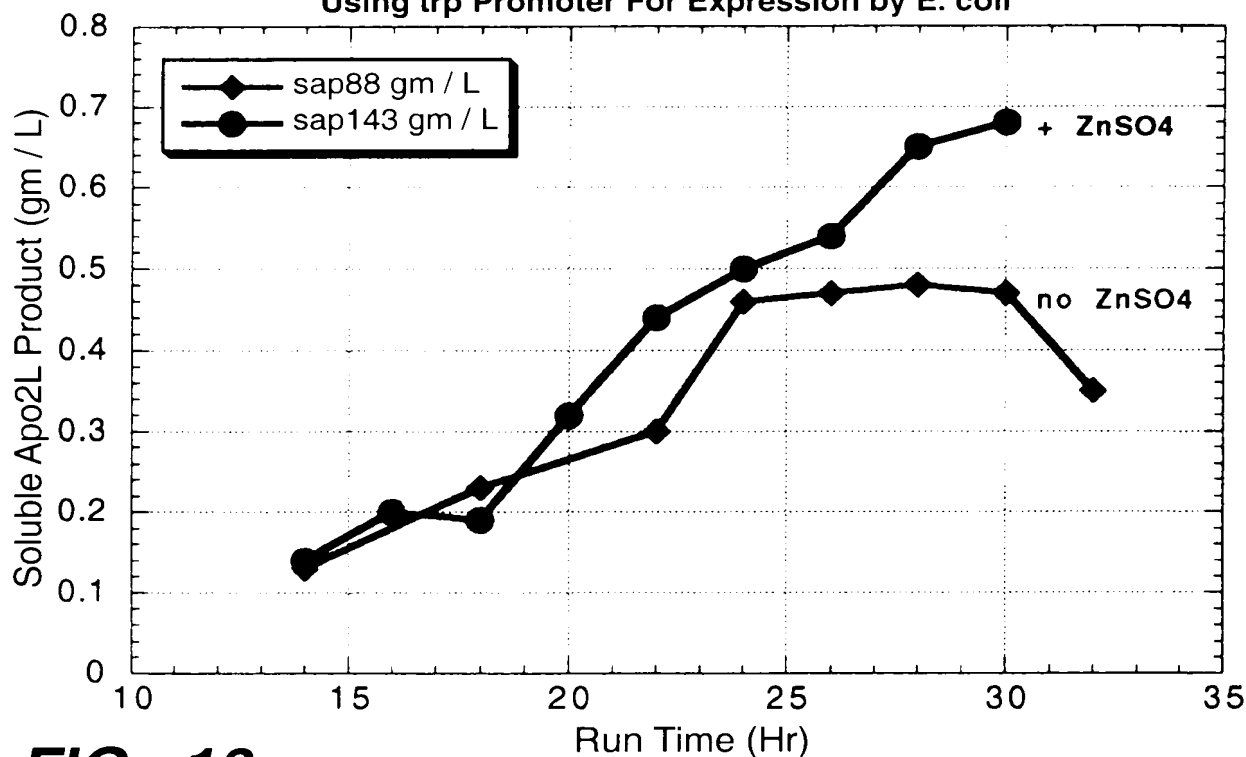


FIG. 10

trp-Apo2L +/- ZnSO₄ gm / L

Effect of CoCl₂ Additions On Apo2L Product Accumulation

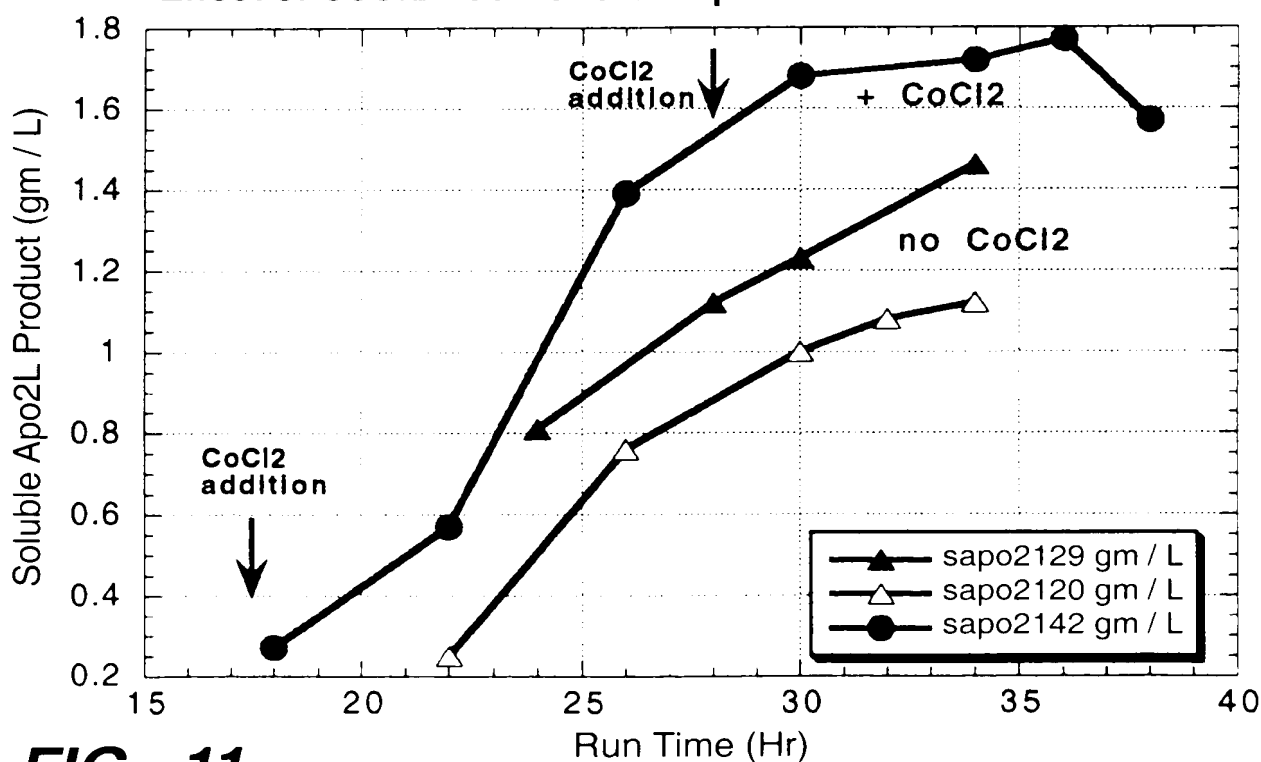


FIG. 11

AP-Apo2L + CoCl₂ gm / L



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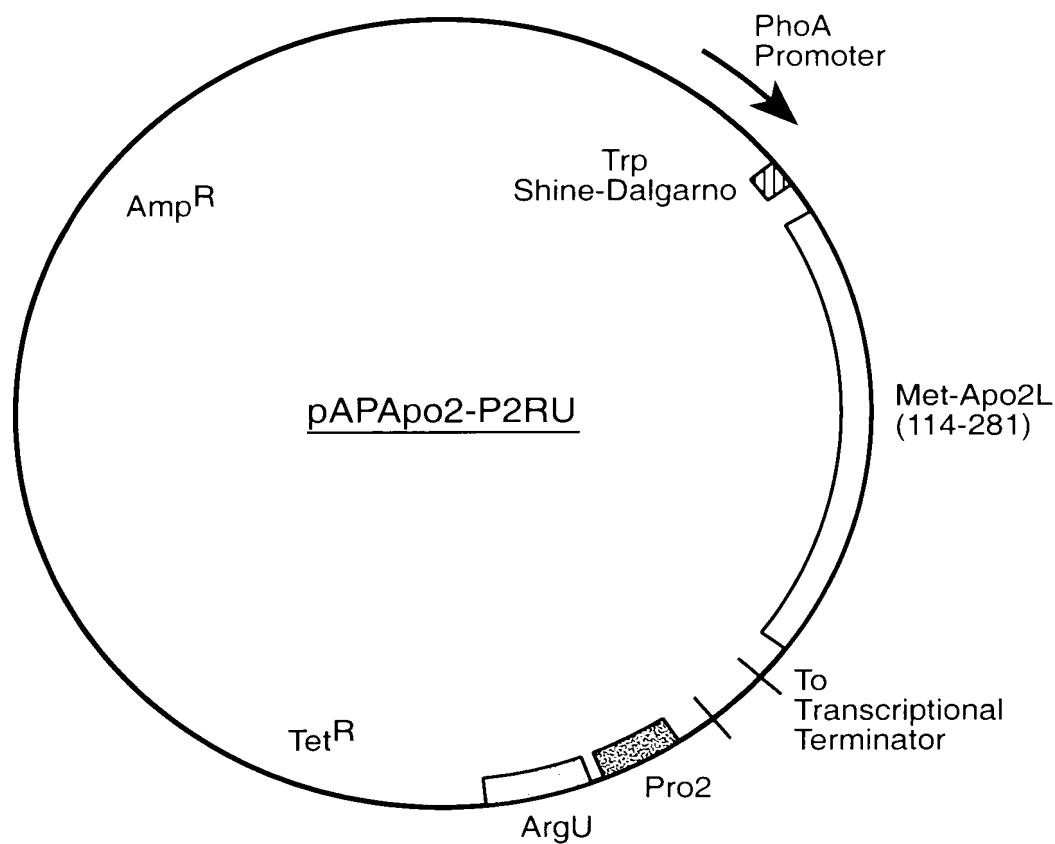


FIG. 12

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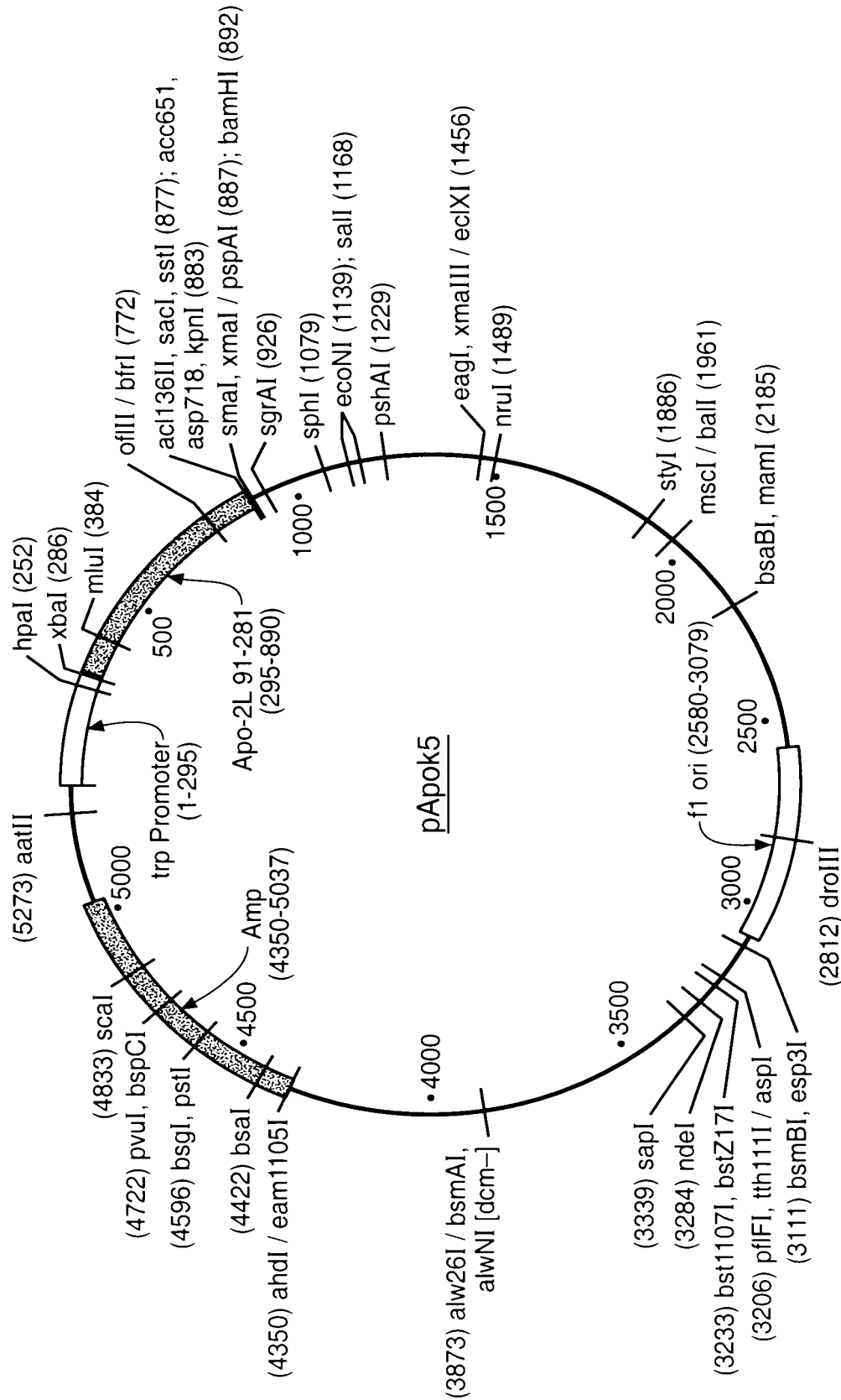


FIG. 13